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Studies on the Symbiosis between Some Natural Truffles and *Taxus wallichiana* Zucc in Lam Dong Province, Vietnam

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Abstract

The truffle gives the formation of the mantle and Hartig net characteristic of the ectomycorrhizal association with host plant roots where water, mineral nutrients and organic carbon are exchanged. Truffles are found in many countries, but are not known in Vietnam. The aim of this research was to examine the effect of some truffles originating from Hungary (*Tuber aestivum, Choiromyces meandriformis and Terfezia sp.)* on *Taxus wallichiana* Zucc development in a greenhouse pot experiment. The mycelia of these fungi were isolated from the original samples on a MMN medium. Six-month old seedlings of *Taxus wallichiana* Zucc were used as test plants for inoculation with fungus mycelia. Pots 250mm in diameter were filled with a 2:1:1 mixture of local dry natural soil, perlite and peat, and each was planted with one plant.
The results showed that *Tuber aestivum* had the better effects on the researched plants growth than *Terferzia* and *Choiromyces meandriformis* both in the diameter and the length development as well as buds forming of the plant. In Vietnam selected natural arbuscular mycorrhizal (AM) fungi helped plant growing as well, while *Trichoderma* spp. reduced this effect.

Keywords: Tuber aestivum, Choiromyces meandriformis, Terfezia, Taxus wallichiana Zucc, plant development, ectomycorrhiza

1. Introduction

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Truffles have been found in Europe, Asia, North Africa, and North America, but not found in Vietnam. They live in close mycorrhizal association with the roots of specific trees. Their fruiting bodies grow underground. The mycelia of truffles form symbiotic relationships with the roots of several host tree species. Colonisation of the host root is the initial step in the symbiotic phase and involves the formation of the mantle and Hartig net characteristic of the ectomycorrhizal association where water, mineral nutrients and organic carbon are exchanged (Harley and Smith, 1983; Chevalier *et al.* 2001).

In Vietnam, there are many kinds of microorganism using to improve the quality of the soil, but the truffle is not found yet in the nature. Till now there are no researches about this fungus. Our study addresses the ability of association between some truffles and *Taxus wallichiana* Zucc in greenhouse, and we have expected of finding the most suitable truffle for *Taxus wallichiana* Zucc growing.

2. Materials and Method

Research place was in Lam Dong province. This province has a tropical monsoon climate and is located in the Central Highlands region of Vietnam. The annual average temperature is from 13-23°C and the annual rainfall is range from 1600 to 2700mm. The soil is clayey and acid.

2.1. Materials

Plants: Six-month-old seedlings of *Taxus wallichiana* Zucc were obtained from a commercial plant centre in Vietnam and transferred to a glasshouse of our Institute in Lam Dong province, in May 2010, and they were chosen randomly for the colonisation experiment.

The fruiting bodies of *Tuber aestivum*, *Choiromyces meandriformis, Terfezia* for the inoculation were transported from the University of Pannonia, Hungary. The mycelia of these fungi were isolated from the original samples on MMN medium.

T.wallichiana Zucc. has a natural symbiotic fungus-partner as well. These arbuscular mycorrhizae (AM), isolated from the plant roots were also included in the examinations.

For studying the protective effect of the examined natural fungus, *a Trichoderma* sp. was also involved in the experiment, which was received from the IBF laboratory of Ho Chi Minh University of Industry.

Soil used in the spot experiment was a 2:1:1 mixture of dry natural soil, perlite and peat. This natural soil was sieved by a 4 mm mesh on site, then steam pasteurized twice at 80^{o}C and air-dried. Ground limestone (<1 mm mesh) was applied to set to pH 7.5, as natural truffles prefer soils containing lime. Plastic pots (25 cm in diameter) filled with equal amounts of the soil mixture were used for plant growing. Each pot was planted with one plant.

2.2. Experimental design and analysis

The green house experiment was carried on as a completely randomized design with 4 inoculation treatment (uninoculated, natural truffle, mycelia of truffle and mix form of truffle with natural and mycelia of truffle*),* 4 kinds of formula (uninoculated, different formula of truffle*;* different formula of truffle was mixed with selected symbiotic fungus of the host plant (AM)*;* and different formula of truffle was mixed with selected symbiotic fungus of the host plant (AM) and *Trichoderma*), and 3 kinds of truffle (*T. aestivum*, *C. meandriformis, Terfezia*) with 15 replicates of individual treatments giving total of 540 pots.

The plants were measured the length, bud forming, diameter by time. The symbiotic connections between plants and microorganisms were investigated via microscope.

The design allowed the unvaried General linear Model (GLM) ANOVA to be performed on all sets of

measured variables. Where significant differences were identified, LSD multiple range tests were performed to identify differences ($P \geq 0.05$).

Table 1. The formulas and signs of experiments using in the research.

Kinds of truffle	Experiments	Form of Truffle			Non truffle (T0)
		Natural (T1)	Mycelia (T2)	Mix (Natural and Mycelia) (T3)	
Non truffle	Non microorganism				T0CT
	Mix with selected symbiotic fungus (AM)				T0AM
	Mix with *Trichoderma* (Tr)				T0Tr
T.aestivum	Control (CT)	T1TaCT	T2TaCT	T3TaCT	
	Mix with selected symbiotic fungus (AM)	T1TaAM	T2TaAM	T3TaAM	
	Mix with AM and *Trichoderma* (Mx)	T1TaMx	T2TaMx	T3TaMx	
Terfezia	Control (CT)	T1TeCT	T2TeCT	T3TeCT	
	Mix with selected symbiotic fungus (AM)	T1TeAM	T2TeAM	T3TeAM	
	Mix with AM and *Trichoderma* (Mx)	T1TeMx	T2TeMx	T3TeMx	
C. meandriformis	Control (CT)	T1CmCT	T2CmCT	T3CmCT	
	Mix with selected symbiotic fungus (AM)	T1CmAM	T2CmAM	T3CmAM	
	Mix AM and *Trichoderma* (Mx)	T1CmMx	T2CmMx	T3CmMx	

3. Results and discussion

3.1. The effect of truffles for the rate of survival T. wallichiana Zucc *plants during the experiment*

In the first 3 months, many plants from experiment have died. From the treatments most plants (75%) died in the group which was treated with *Terfezia* and arbuscular mycorrhizae (AM). In some cases, when *Terfezia* or *C. meandriformis* was mixed with AM and *Trichoderma,* the amount of the dead plants was high (62.5-64.3%) as well. However in case of the treatments without any colonization, the number of dead plants always was among the highest (72.7%). The dead plants were replaced with new plants. After the third replacement of the dead plants, the rate of death was reduced. This result could be caused by the use of lime to raise soil pH to neutral, while *T. wallichiana* Zucc is living in the acid soil. However, after time, this plant has adapted to the new environment with the help of the truffles symbiozis. The plants which were treated with *T.aestivum* were adapted more quickly than with *C. meandriformis* and *Terfezia sp*.

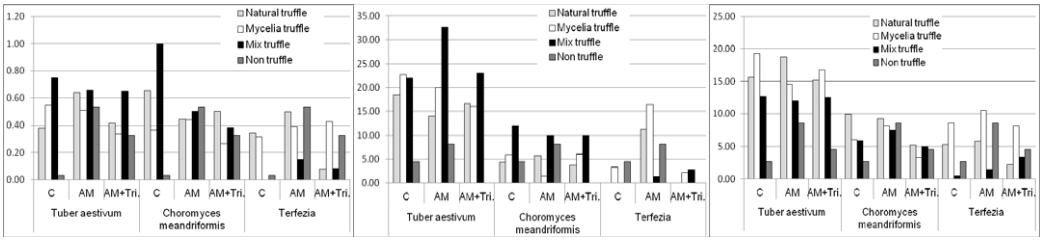
3.2. The effect of truffles on the stem diameter of T. wallichiana Zucc.

After 9 months in the treatments, with different kinds of truffle the results were different. In usual the diameter of the *T. wallichiana* Zucc seedlings inoculated with different truffles grew faster than those which were not treated with truffle (Fig.1a). The best result was performed when the mixed form of *C.meandriformis* was used (mixture the natural and mycelia truffle with different fungus) (Fig 1a).

When the samples were mixed with *T. aestivum*, the development of the stem diameter of *T. wallichiana* Zucc was better in every case. We could observe that in the treatments without *T. aestivum* inoculation the stem diameter of the seedlings *T. wallichiana* Zucc developed slower than those inoculated with the *T. aestivum*. The result was the best when the mix form of *T.aestivum* was used (Fig 1a).

Using sole *C.meandriformis* for inoculation, it positively affected the development of *T. wallichiana* Zucc as well. However, in the case *C.meandriformis* was mixed with AM or AM and *Trichoderma*, the diameter of *T. wallichiana* Zucc was nearly the same, and it did not differ from the data of the treatment without

C.meandriformis. Compared to the control (no truffle), *Terfezia* didn't affect the development of *T. wallichiana* Zucc, except, it was mixed with AM or AM and *Trichoderma*. When mixed with the latter fungus, *Terfezia* increased stem diameter.



(a) (b) (c)

Fig.1. Effect of truffle on *T. wallichiana* Zucc development: a) comparison of plants diameter differences (mm) and b) the plants height differences (mm) c) the amount of buds forming

3.3. The effect of truffles on the stem elongation of T. wallichiana Zucc

Comparing the impact of the three truffle species, we can see that *T.eastivum* effected much higher the stem elongation of *T.wallichiana* Zucc than the other two species (Fig. 1b).

After inoculation with the mixed form of *T.eastivum* (T3TaCT), the average of plants length was 22.0 mm longer than the initial plants height (262.4mm). In the case, if plants were inoculated with the mixed form of *T.eastivum* and AM (T3TaAM) plant height was also 32.7 mm taller than the early samples (216.7mm). However, *Trichoderma* reduced the impact of AM on the plant development. After treatment with the mixed form and AM and *Trichoderma* (T3TaMx) the plant was only 23.0 mm longer than the beginning (231.8mm) (Fig.1b). Nevertheless, the other formula of treatment with *T.eastivum* Zucc gave a better effect on the plant growing as well, but it was not as positive as the mixed form.

C.meandriformis and *Terfezia* did not much affect on stem elongation of *T.wallichiana* Zucc. When *C.meandriformis* was used, as a mixed form, it also gave the best result (T3CmCT, T3CmAm, and T3CmMx).

The effect of *Terfezia* was much slighter. When it was mixed with AM, the plant height development was faster. Possibly this effect was more caused by the AM, than by the *Terfezia sp*. So it must be tested with microscope to see the symbiotic form.

3.4. The effect of truffles to the bud forming of T.wallichiana Zucc *during tree growing*

The number of forming buds of *T. wallichiana* Zucc was higher in the case of all plants which were treated with *T.aestivum*, than without. On the Fig.1c, can be seen that plants which were treated with pure truffle or with mixture of *T.aestivum* and AM fungus had a higher number of forming buds, than in case if it was treated with *Trichoderma*.

Seedlings inoculated with *T.aestivum*, originating from Hungary, and with AM, the in Vietnam selected symbiotic fungus, produced the best result for *T.wallichiana* Zucc development both on the plant height and buds forming. This result indicates a differential effect of Vietnamese selected symbiotic fungus and *T.aestivum* on *T. wallichiana* Zucc growth.

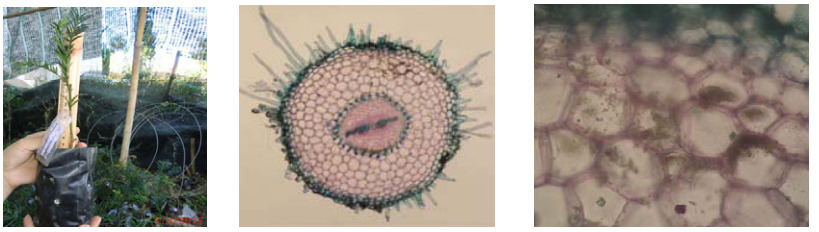
The samples which were treated with only *C.meandriformis*, was forming more buds, than the non treated samples. However when the soil was mixed with AM and *C.meandriformis*, the amount of the forming buds was nearly similar in all the samples. This way, AM had a positive effect on the buds forming. In the case of using the mixture of AM and *Trichoderma*, the results were similar to it, if AM was used, but the amount of

the forming buds was less. Presumably, because *Trichoderma* influenced AM negatively.

Terfezia gave not as much forming buds as *T. aestivum*. The formula which was used mycelia form of *Terfezia* had the best effect in all cases. AM also had a good effect on the buds forming, and *Trichoderma* also had a negative effect on AM to form new buds.

After 9 months treatment each truffle had different effect on buds forming of *T.wallichiana* Zucc. *T.aestivum* gave the best effect, while *Terferzia* gave the least. In the formula which AM was used the results were better then the others. Meanwhile *Trichoderma* affected worse on AM.

3.5. Compare the symbiotic states between studied truffle during T. wallichiana Zucc *development*



(a) (b) c)

Fig. 2. a) the researched plants; b) the root of plant after 6 moths infection with truffle; c) the truffle was infected inside root

The result showed that all of researched truffle made the infection in the *T. willichiana* Zucc, but not the same way. *T.aestivum* affected on the researched plant more than others. *Terfezia* was infected less. After treatment in the green house, all reached tree must transfer to the forest. The environment could have more affection on the plant growing.

4. Conclusion

This study was considered the effect of three kinds of truffle (*Tuber aestivum, Choiromyces meandriformis and Terfezia sp.)* on *T.willichiana* Zucc. The results showed that different kinds and different state of truffles responded differently to addition fungus in partnership with host plants. So it can be concluded that *T.aestivum* gave better effects to *T. wallichiana* Zucc growth than the others in the tropical climate. It also can be seen that AM gave good effect to this tree. Meanwhile in some cases, *Trichoderma* gave bad effect to AM.

The continued research should be considered not only the effect of truffles on the other host plants but also the ability of fruit forming from this truffle. So the new valuable product can be produced in the future.

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